The relation of this latter pathway to the mature fibrils is not entirely clear. Intriguingly, it is these latter oligomers and protofibrils that have been implicated as the molecular species mediating the cellular toxicity associated with amyloid diseases.
For the amyloidogenic protein lysozyme, we have systematically mapped out the combination of protein and salt concentrations resulting in the formation of either long rigid or oligomeric amyloid aggregates for fixed temperature and pH. Using dynamic light scattering, thioflavin fluorescence spectroscopy, atomic force microscopy and infrared spectroscopy, we detected three distinct types of aggregates. Growth of long straight fibrils prevailed at either low salt or protein concentrations. At intermediate salt and protein concentration oligomer formation with subsequent protofibril nucleation prevailed. Oligomers and protofibrils represent metastable phases that are kinetically favored, while long straight fibrils are the thermodynamically stable state. Eventually, fibril formation gives way to amorphous precipitation. This phase behavior shows intriguing similarities with the phase diagram for protein crystallization where a metastable liquid-liquid phase is located within the stable coexistence region for protein crystals.

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Polyglutamine Flanking Regions in Huntingtin Highlight Key Structural Intermediates Relevant for Molecular Chaperone Interaction and Huntington's Disease Pathogenesis
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Huntington's Disease (HD) is a neurodegenerative disorder caused by an expansion in the polyglutamine (polyQ) tract of the Huntingtin (Htt) protein. The clinical hallmark of this mutation is the accumulation of amyloid Htt aggregates in neurons. While Htt aggregation is highly correlated with the length of this polyQ tract both in vitro and in vivo, recent studies suggest that the regions flanking the polyQ tract can influence Htt aggregation and toxicity independent of polyQ length. In addition, it is well established that two polyQ flanking regions in exon1 of Htt, the N-terminal first 17 amino acids (N17) and a proline-rich region (PolyPro), influence Htt aggregation propensity. Here, we show that the N17 and PolyPro regions of Htt dramatically influence the rate of the Htt aggregation pathway. We also show that mutations within these polyQ-flanking regions alter Htt toxicity in a brain slice model. The influence of these flanking regions on the heterogeneous distribution of the aggregate species may account for these differences in toxicity. This theory is increasingly relevant in light of recent thought that Htt toxicity may derive from a species other than the amyloid fibril itself. Finally, previous work has shown that the N17 region of Htt interacts with the molecular chaperonin TRiC, and this interaction exerts a protective effect against both Htt aggregation and toxicity. An understanding how these flanking regions influence Htt aggregation will inform how TRiC works through the N17 element. Together, this information can provide the basis to design HD therapeutics that exploit these flanking regions to suppress HD pathogenesis.

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Kinetics of the Interconversion Between Two Physiologically Important Copper-Bound Amyloid-Beta Species
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To better understand the physiological behaviour of Aβ in the brain, the kinetics of the interactions between metal ions and Aβ is crucial. In 1:1 stoichiometry binding between cooper ions and Aβ, two major conformations have been characterized in equilibrium in literature. We found that Aβ binds to a first copper ion with a near diffusion limited rate constant ~10 ^ 8 M ^ {-1}s ^ {-1}, to a second copper ion at a rate constant ~10 ^ 5 M ^ {-1}s ^ {-1}, and further copper ions. The reaction between EDTA and a mixture of 50 nM Aβ and various amounts of copper ions ranging from nM to 10 μM shows the evolution of multiple copper-Aβ species as a function of copper concentration. Most interestingly, the ratio of the two major species at low copper concentrations depends on EDTA concentration, suggesting the interconversion between them, with the rates in the order of s ^ {-1}. Whether this interconversion is relevant to the roles of Aβ in health and disease is unclear.

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Insights into the Inhibition Mechanism of Biomolecular Self-Assembly from Chemical Kinetics
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Understanding and control the aggregation of biomolecules at the molecular level can open attractive possibilities to correct dysfunctional cell behaviour. For instance, the inhibition of protein aggregation is emerging as a potential attractive therapeutic strategy against several neurodegenerative disorders. For the development of successful treatments, it is crucial to achieve a controlled intervention on specific toxic species. In this perspective, an understanding of the molecular inhibition mechanism of protein self-assembly is of fundamental importance but remains challenging to achieve.
In this work, we demonstrate how chemical kinetic analysis can be applied to elucidate the molecular mechanism of inhibition of several classes of compounds such as small chemical molecules, nanoparticles, peptides and proteins. By applying a population balance model we show how it is possible to obtain information on the specific inhibited microscopic event and on the specific protein target species responsible for this inhibition. We demonstrate the potentiality of the approach by analyzing the inhibition mechanism of selected chaperones, protein regulators of the proteostais network and relevant naturally occurring inhibitors of protein aggregation, on the aggregation of a yeast prion protein and of Abeta42, the peptide involved in Alzheimer's disease. In addition, we discuss relevant implications of the controlled inhibition of protein aggregation in the engineering of the fibrillation reaction pathway and in the development of effective therapeutic strategies.

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Filament Assembly by Phosphofructokinase-1, the Gatekeeper of Glycolysis
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The cytoskeleton is conventionally viewed as being composed of three filamentous networks; microfilaments, microtubules, and intermediate filaments. This view is challenged by the findings that metabolic enzymes can form filaments with structural functions. We report that phosphofructokinase-1 (PFK1), the first rate-limiting step of glycolysis, assembles into filaments in vitro and in cells. Transmission electron microscopy (TEM) showed that purified liver PFK1 is mainly tetrameric and occasionally formed short filaments in the absence of substrate. Adding the substrate fructose 6-phosphate (F6P) induced the assembly of predominantly long filaments measuring up to 250 nm. PFK1 filaments were less rigid than actin polymers, displaying right angles in contiguous assemblies. The filaments were composed of individual tetramers and had a uniform 11 nm width, resembling an organized addition of subunits forming polymers. Regulated assembly into filaments was also indicated by light scattering measurements that showed a rapid substrate-dependent increase in scattering followed by a stable plateau. Increased light scattering was blocked by excess ATP, which inhibits PFK1 activity. To further confirm activity-dependent filament assembly we generated an inactive but tetrameric liver PFK1 mutant, His199Tyr, and found that in the presence of F6P it does not form filaments, as determined by TEM, or show an increase in light scattering. To assess filament formation by PFK1 in cells, we expressed GFP-tagged PFK1 and used live-cell imaging to examine GFP-PFK1 dynamics. Confocal microscopy reveled that cytosolic PFK1 was recruited to the distal margin of lamellipodia that were devoid of mitochondria. TIRF microscopy reveled that GFP-PFK1 formed dynamic punctae. These data indicate that active but not inactive PFK1 assembles into tetramer-aligned filaments. The activity-dependent recruitment and assembly of PFK1 filaments at the plasma membrane could provide a scaffolding and structural framework for localized ATP production in lamellipodia that lack mitochondria.

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Amyloid β-Protein: The Influence of Intrinsic and Extrinsic Factors on Fibril Formation
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Aggregation of the amyloid β-protein (Aβ) is believed to be involved in Alzheimer's disease pathogenesis. The central hydrophobic region (CHR) and the Aβ42/Aβ40 ratio play key roles in Aβ aggregation. Studying intrinsic (amino acid substitutions) and extrinsic (temperature, other molecules) factors contributes to understanding the mechanisms that cause Aβ monomers to aggregate and form oligomers and fibrils. This could facilitate the development of agents that therapeutically target toxic assemblies or prevent their formation.
In our studies we mainly used a highly reproducible thioflavin T assay to probe the aggregation kinetics. Substitution of phenylalanine with leucine at position